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# ENDOTOXIN INDUCED ENDOGENOUS MEDIATORS IN THE PATHOGENESIS OF SEPTICAEMIA

DAAN W. HOMMES[1], YONGQIANG ZHAO[2], ENRICO BERNARDI[3], JAAP JANSEN[1], SANDRA A. RADEMA[1], and SANDER J.H. VAN DEVENTER[1,*]

[1]Centre for Haemostasis, Thrombosis, Atherosclerosis and Inflammation Research, Academical Medical Centre, Amsterdam, The Netherlands; [2]Department of Haematology, Peking Union Medical College Hospital, Beijing, China; and [3]Department for Thrombosis and Haemostasis, University of Padua, Italy.

## SUMMARY

Endotoxin is a major causal Gram-negative bacterial factor in the development in Gram-negative septicaemia. The bowel contains a vast amount of endotoxin, that is derived by shedding of outer membrane fragments of either growing or dead bacteria. Normally, very little endotoxin is absorbed by the gut. In animal models, conditions that damage the mucosal lining, such as ischaemia/reperfusion, profound metabolic changes, or bowel inflammation, may cause transmigration of endotoxin. In humans, similar conditions, including bowel ischaemia and extensive inflammatory bowel disease, may similarly lead to "intestinal endotoxaemia". Some investigators have proposed that continuous endotoxin uptake may be an important causative factor in the development of multiple organ failure. Although it is possible that endotoxin uptake through damaged bowel mucosa has a role in the perpetuation of septicaemia in critically ill patients, there are currently no data to either substantiate or falsify this hypothesis .

Despite improvements in the management of critically ill patients, the mortality of septicaemia remains high. Immunotherapy, in particular treatment with cross-reactive anti endotoxin (glyco)lipid antibodies, may improve survival in septic patients with Gram-negative bacteraemia. Clinical studies with antibodies that neutralise an important endogenous mediator of endotoxicity, tumour necrosis factor, are underway, and may show additional benefit.

## INTRODUCTION

Gram-negative septicaemia remains a major cause of death among hospitalised patients and is the commonest cause of death in intensive care units. In the United States the annual incidence of septicaemia is estimated to be 400.000, leading to 200.000 cases of septic shock and 100.000 deaths (*Parrillo* et al., 1990). Gram-negative septicaemia is caused by bacterial products, such as endotoxins, that enter the blood stream and trigger a series of reactions including complement activation (via both the classical and alternative pathways) (*Vukajlovitch* et al., 1987), platelet activation (*Doebber* et al.,

*Dr. van Deventer is a fellow of the Netherlands Academy of Arts and Sciences.

1985), activation of the coagulation and fibrinolytic system (*Harris* et al., 1987) and the induction of the synthesis and release of various cytokines from mononuclear and endothelial cells (*Tracey* et al., 1986; *Michie* et al., 1988; *van Deventer* et al., 1990; *Fong* et al., 1989a; *Girardin* et al., 1988; *Hack* et al., 1989).

Endotoxins are lipopolysaccharides (LPS) from the Gram-negative bacterial cell wall. Endotoxin is composed of three portions: the oligosaccharide side chain, the core polysaccharide and the lipid A component. Lipid A is structurally similar among many Gram-negative bacteria, and it represents the biologically active part of the endotoxin molecule, being responsible for the toxic properties of endotoxin (*Appelmelk* et al., 1988; *Rietschel* et al., 1982). Naturally occurring antibodies to lipid A are associated with increased survival in human septicaemia (*McCabe* et al., 1972), and a recent clinical trial with a human monoclonal anti-endotoxin antibody HA-1A, which binds to the lipid A component, showed a significant reduction of mortality in patients with septicaemia (*Ziegler* et al., 1991).

In the last decade, extensive research has given us considerable insight into the interplay of endotoxin with the immune system. It has become apparent that almost all toxic effects of lipid A are mediated by endogenous (glyco)proteins, called cytokines, that are released by monocytes and endothelial cells. Here we briefly review the role of endotoxin and cytokines in septicaemia, and discuss novel therapeutic approaches in Gram-negative septicaemia, with special emphasis on gut derived endotoxin as a cause of septicaemia.

## CYTOKINES

The normal human immune response is regulated by cytokines, polypeptide hormones that are synthesised and release by various cell-types. Cytokines regulate the biological function of various tissues by modifying gene expression and cellular metabolism. Many cytokines have been molecularly cloned and recombinant proteins are available to investigate their role in immune responses and inflammation. Although each cytokine has a distinct sequence, structure and individual receptors, cytokines often share similar biological properties, and multiple cytokines can act synergistically or antagonistically on the same target cells. Although cytokines were first identified in monocytes and macrophages, it is now clear that many cytokines can be produced by other cells, including those that are traditionally not considered to be "immunocompetent": Fibroblasts, endothelial cells, bone marrow, epidermal keratinocytes, stromal cells and brain astrocytes are able to produce substantial amounts of cytokines in response to various stimuli.

Cytokines can be divided into two main groups: I. cytokines that predominantly function as *growth factors* (interleukin-1 [IL-2], interleukin-3 [IL-3], interleukin-4 [IL-4]) and II. cytokines that predominantly have *pro-inflammatory properties* (interleukin-1 [IL-1], interleukin-6 [IL-6], interleukin-8 [IL-8] tumour necrosis factor [TNF]). IL-2 and IL-4 stimulate the growth and functional activities of T and B lymphocytes, and IL-3, together with granulocyte-macrophage colony stimulating factor (GM-CSF), is a growth and progression factor for haematopoietic cells. The pro-inflammatory cytokines (IL-1,

IL-6, IL-8, TNF) have been extensively studied and are now known to have a pivotal role in the pathogenesis of septicaemia. In the context of a normal immune response these cytokines are produced in very small amounts (usually below the detection limits in serum), necessary for a normal host defence response. In septicaemia however, uncontrolled cytokine release may cause hypotension, organ damage, catabolism, and death.

## INTERLEUKIN-1

IL-1 was one of the first endogenous pyrogens to be identified, and has a large variety of other effects including the induction of PGE2 synthesis, growth of fibroblasts, bone resorption, expression of cell adhesion molecules, sleep, anorexia, synthesis of collagenase and growth and differentiation of T and B cells (*Dinarello*, 1988). In intestinal disease states, IL-1 is also responsible for an increase of intestinal mucus production (*Han* et al., 1987), which may constitute a normal host defence response. In animal models of experimental colitis, IL-1β is predominantly produced by the enterocytes of the colonic mucosa (*Radema* et al., 1991), suggesting a central role of IL-1 in inflammatory bowel disease.

Two separate IL-1 molecules have been cloned, IL-1α and IL-1β, each one encoded by a separate gene on chromosome 2 (*Lomedico* et al, 1984; *Auron* et al., 1984). Although the two forms only share a 26% amino-acid homology, they have similar activities and bind to the same cellular receptor (*Urdal* et al., 1988). The administration of IL-1 to animals induces several systemic changes typical of inflammatory reactions, including fever, neutrophilia, synthesis of hepatic acute phase proteins, hypotension and increased corticosteroid production (*Dinarello*, 1989). In contrast, pretreatment with low doses of recombinant IL-1β (30-300 ng/kg) protects against death and tissue injury in animal models of Gram-negative infection (*van der Meer* et al., 1988), radiation (*Neta* et al., 1986), contact hypersensitivity (*Robertson* et al., 1987), arthritis (*Jacobs* et al., 1988) and hyperoxia (*White* et al., 1987). One explanation for these protective effects is an increase of endogenous prostaglandin levels (Cominelli et al., 1990).

## TUMOUR NECROSIS FACTOR

A century ago, infection-induced haemorrhagic necrosis of tumours was described in man by *Coley* (1983). It took almost a century until the responsible factor, tumour necrosis factor (TNF, also known as cachectin), was independently isolated by two groups of investigators (*Aggarwal* et al., 1985; *Beutler* et al., 1985a; *Beutler* et al., 1985b). TNF is predominantly produced by monocytes and macrophages and has a still growing list of biological properties, including activation of polymorphonuclear leukocytes (PMN), increasing leukocyte/endothelial cell adhesion, cachexia, inhibition of lipoprotein lipase, tumoricidal activity, bone resorption, and procoagulant activity (*Sherry* and *Cerami*, 1988; *Tracey*, 1989; *Beutler* et al, 1985a; *Tsujimoto* et al., 1986). TNF was found to be produced in large quantities by some tumour cells, including the myelomonocytic cell line RAW 264.7 from which it was initially

isolated (*Beutler* et al., 1985a; *Mahoney* et al., 1985). TNF is encoded by a gene located on chromosome 6 and is synthesised as a 232 amino-acid peptide. Cleavage of a relatively long propeptide yields the secreted mature hormone (*Jones* et al., 1989). In volunteers, circulating TNF can be detected 30 minutes after endotoxin administration, reaching a peak at 90-120 minutes (*Jones* et al., 1989; *Michie* et al., 1988). In experimental animals, high doses of TNF cause a syndrome which is indistinguishable from septic shock, characterised by hypotension, metabolic acidosis, haemorrhagic infarction of the gastrointestinal tract, and release of catecholamines and glucocorticoids (*Tracey* et al., 1986). Sustained exposition to low and chronically administered doses of TNF causes a profound catabolic state that resembles the metabolic changes that are observed in patients with chronic inflammatory diseases, including anorexia and wasting, suppression of lipoprotein lipase (*Tracey* et al., 1987; *Oliff* et al., 1987), and a marked resorption of fat.

## INTERLEUKIN-6

IL-6 was first detected in T cell supernatants as a helper T factor that was observed to induce immunoglobulin secretion by B cells (*Schimple* and *Wecker*, 1972; *Kishimoto* and *Ishizaka*, 1973). IL-6 is a potent inducer of the expression of acute phase proteins by hepatocytes (*Castell* et al., 1989), and has the capability of activating B and T lymphocytes. In addition, IL-6 causes haematopoietic stem cell growth, maturation of megakaryocytes, neural cell differentiation, mesangial cell growth and myeloid leukaemic cell differentiation. The human IL-6 gene is located on chromosome 7, and the secreted protein is glycosylated. A wide variety of cells may produce IL-6, but it is likely that in septicaemia macrophages and endothelial cells are its main source.

In summary, IL-6 has emerged as a major systemic acute signal-peptide which elicits a variety of host defence responses of the host.

## INTERPLAY OF CYTOKINES

There is accumulating evidence of considerable interplay between IL-1, TNF and IL-6, and some of their functions overlap. TNF and IL-1 may reciprocally stimulate their synthesis *in vitro* as well as *in vivo* (*Le* and *Vilcek*, 1989) and both cytokines trigger the production of IL-6 (*Le* and *Vilcek*, 1989). In contrast, IL-6 impairs LPS-induced TNF and IL-1 production by mononuclear cells *in vitro* (*Aderka* et al., 1989; *Schindler* et al., 1990). Furthermore, IL-1, IL-6 and TNF all are endogenous pyrogens and trigger the liver to express genes for acute phase proteins. Both IL-1 and TNF, but not IL-6, induce the production of cycloxygenase and phospholipase $A_2$. Corticosteroids suppress both the transcription and translation of IL-1 and TNF as well as that of other cytokines. Both TNF and IL-1 are responsible for the recruitment of neutrophils to inflammatory sites by activation of several mechanisms. The first step in neutrophil recruitment is adhesion to endothelial cells, which is reflected by a transient precipitous fall in circulating neutrophil numbers in experimental endotoxaemia in humans (*van Deventer*, 1988). Both IL-1 and TNF

elaborate rapid expression of the adhesion molecules ELAM-1, GMP-140 and ICAM-1 on endothelial cells (*Bevilacqua* et al., 1989) that are involved in binding of neutrophils. These cytokines also cause expression of receptors, such as VCAM-1 and the VLA integrins, that mediate lymphocyte adhesion to endothelial cells (*Stoolman*, 1989).

## NOVEL ENDOTOXIN-INDUCED CYTOKINES

Although many of the host-immune responses and inflammatory changes during septicaemia can be attributed to IL-1, IL-6 and TNF, it has become clear that other cytokine hormones participate in the overall host-defence response. Macrophages, when stimulated with LPS, release a multitude of induced proteins, many with yet undefined structures and functions (*Nathan*, 1987). Some biological activities that were previously ascribed to known cytokines may in fact result from the release of other mediators. For example, it has become clear that both *in vivo* neutrophil chemo-attractive potential of TNF and the neutrophil activating capability of IL-1 result from the induction of secondary protein mediators such as IL-8. IL-8 is a member of a class of small cytokines (SCY-family) that have similar cDNA sequences and genomic structure. Other small cytokines which belong to this SCY-family and which are now identified are the murine macrophage inflammatory proteins 1 and 2 (MIP1, MIP2), platelet factor 4 (PF4) and monocyte chemo-attractant protein 1 (MCP1). Most of these cytokines have only been recently identified and many of their biological properties remain unknown. It seems that IL-8 is importantly involved in the pathogenesis of septicaemia. IL-8 is generated by macrophages and endothelial cells after stimulation by LPS, TNF or IL-1, and is chemotactic for neutrophils, basophils and T cells but not for monocytes. In addition it is a potent stimulator of neutrophils. In rats, injection of recombinant IL-8 caused extensive recruitment and accelerated migration of neutrophils across high endothelial venules (*Larsen* et al, 1989).

## GUT-DERIVED ENDOTOXINS IN SEPTICAEMIA

In critically ill patients endotoxins may transmigrate the gut mucosal lining, and cause additional activation of mediator systems. Surgical manipulation may further contribute to endotoxin release from the bowel. Endotoxaemia itself impairs glutamine metabolism, thereby causing a breakdown of the gut mucosal barrier (*Souba* et al., 1990), and "bowel rest" during total parenteral nutrition, results in enhanced endotoxin-induced splanchnic cytokine responses (*Fong* et al., 1989b). Thus, in various conditions in which critically ill patients are unable to tolerate enteral feeding, "intestinal endotoxaemia" may be an important pathophysiological entity (*van Deventer* et al., 1988a), and in this context the gut has been named the "motor of MOF" (multiple organ failure). We here briefly review studies on the transport of intestinal endotoxins and the mechanism and pathogenesis of endotoxin absorption.

## DOES THE NORMAL BOWEL ABSORB ENDOTOXIN?

Large amounts of endotoxin are present in the gut, and even in germ-free animals the intestinal endotoxin concentration is substantial (*Rush* et al., 1989). A major point of dispute remains to what extent the normal intestine absorbs endotoxin. Early experimental studies showed an increase in radioactivity in the liver of rabbits (not in other organs) that were fed $^{32}$P O111;B4 lipopolysaccharide (*Ravin* et al., 1960). However, no substantial transport of $^{51}$Cr labelled bacterial lipopolysaccharide through the intact bowel could be detected (*Sanford* and *Noyes*, 1958). These results should be interpreted cautiously because radioactive label can dissociate from endotoxin, thereby causing false positive results. Using immunohistochemistry, lipopolysaccharides that were instilled into the proximal large bowel of normal rats were not detected in the bowel wall, nor in intestinal lymph nodes, peritoneal cavity, or liver sinusoids (*Schoeffel* et al., 1989) .

It has been the traditional view that endotoxins are normally present in human portal blood (*Pain* and *Bailey*, 1987; *Tachiyama* et al., 1988; *Lumsden* et al., 1988). In this view, Kupffer cells in the liver prevent systemic endotoxaemia by removal of endotoxins, which would explain the occurrence of systemic endotoxaemia in liver failure. For two reasons this hypothesis now appears to be incorrect. Firstly, although a high incidence of portal endotoxaemia in normal humans has been reported (*Prytz* et al., 1976; *Jacob* et al., 1977; *Pain* and *Bailey*, 1987; *Tachiyama* et al., 1988), in two recent prospective studies in consecutive patients without diseases of bowel or liver, no endotoxin could be detected in the portal or systemic circulation (*van Deventer* et al., 1988b; *Brearly* et al., 1985). Secondly, it is difficult to explain why Kupffer cells, that are main sources of TNF, IL-1, and other cytokines, do not release these mediators after stimulation with intestinal endotoxin, while these cells release substantial amounts of TNF following systemic administration of small amounts of endotoxin (*Fong* et al., 1989b). Presently, most investigators therefore agree that the intact bowel mucosa provides an effective defence barrier for endotoxin, and that endotoxin transmigration results from damage to the integrity of the bowel mucosa. Consequently, in liver failure, intestinal damage due to portal hypertension, rather than Kupffer cell depression, seems to induce endotoxin uptake from the gut.

## INTESTINAL ENDOTOXAEMIA

Circumstantial evidence implicates many pathophysiological conditions, as potential causes of "intestinal endotoxaemia". Although the precise molecular mechanisms and kinetics of endotoxin transmigration in these circumstances remain to be elucidated, a decreased production of mucus and damage to the integrity of the bowel mucosa, resulting in disruption of tight junctions between mucosal cells, seem to be pathophysiologically important. Here, we briefly summarise clinical and experimental data that provide evidence for a pathophysiological role of gut-derived endotoxin in haemorrhagic shock, intestinal ischaemia, jaundice, bowel obstruction and other conditions.

## Haemorrhagic shock and intestinal ischaemia

In rats, haemorrhagic shock rapidly results in systemic endotoxaemia and bacteraemia (87% and 50% respectively, after 2 hours) (*Rush* et al., 1988). The mucosal damage in haemorrhagic shock and its mechanism has been elegantly studied by *Deitch* and colleagues. Using horseradish peroxidase as a marker, they demonstrated an increased intestinal permeability in experimental haemorrhagic shock, that coincided with the appearance of subepithelial oedema and focal necrosis of the ileal and coecal mucosa. In addition translocation of bacteria, most commonly *Escherichia coli* and *Enterococcus* species, to mesenteric lymph nodes, liver and spleen was observed (*Baker* et al., 1988; *Deitch* et al., 1990a). Interestingly, systemic endotoxaemia itself also caused a significant bacterial translocation accompanied by disruption of mucosal barrier (*Deitch* et al., 1987; 1989a; *Navaratnam* et al., 1990; *O'Dwyer* et al., 1988). It appears that the mucosal damage in haemorrhagic shock is mediated by release of oxidants that are derived from the xanthine oxidase system. Inhibition of xanthine oxidase by oral administration of allopurinol or its inactivation by feeding a tungsten-supplemented molybdenum-free diet, resulted in significant decreases in bacterial translocation (*Deitch* et al., 1988; 1990b). One should keep in mind however that it is likely that bacterial translocation and endotoxin absorption are different processes, that may be induced by different stimuli.

Multiple studies have demonstrated that intestinal ischaemia is rapidly followed by systemic endotoxaemia (*Cuevas* and *Fine*, 1972; *Nozickova* et al., 1977; *Olofsson* et al., 1985). In rats, bowel ischaemia results in subsequent appearance of endotoxin in the thoracic duct, the portal vein, and the systemic circulation (*Olofsson* et al., 1985). Likewise, in dogs with superior mesenteric occlusion, a high endotoxin concentration may be observed (*Nozickova* et al., 1977). Thus in intestinal ischaemia, as well as in other conditions, the thoracic duct appears to be a major route of endotoxin uptake (*Daniele* et al., 1970; *Olofsson* et al., 1986; *Olofsson*, 1988). Disruption of the intestinal barrier in intestinal ischaemia may be caused by oxygen radicals that are formed during ischaemia, and released following reperfusion (*Schoenberg* and *Beger*, 1990).

## Obstructive jaundice

The clinical finding that the incidence of postoperative renal impairment is particularly high in obstructive jaundice (*Bailey*, 1976; *Wilkinson* et al., 1976; *Cahill*, 1983), has prompted clinical and experimental studies on endotoxaemia as a pathogenic factor in this setting. Indeed, jaundiced patients or experimental rats have a high incidence of portal and systemic endotoxaemia (*Wilkinson* et al., 1976; *Cahill* et al., 1987; *Pain* and *Bailey*, 1987; *Blumgart*, 1988; *Thompson* et al., 1988; *van Bossuyt* et al., 1990; *Diamond* et al., 1990). It has been shown that in rats internal bile drainage, but not external drainage, protects against intestinal endotoxin uptake (*Gouma* et al., 1986). Although ligation of the common bile duct in mice leads to subepithelial oedema of the ileal villi and an increase in bacterial translocation (*Deitch* et al., 1990c) and jaundice may cause a depression of the mononuclear phagocytic system (*Drivas* et al., 1976), the absence of bile salts in the intestinal lumen therefore seems to be the pivotal factor for intestinal endotoxin uptake in jaundice. This hypothesis is supported by clinical studies as well as animal exper-

iments that demonstrated a reduced incidence of postoperative endotoxaemia and a decreased occurrence of postoperative complications after oral administration of bile salts (*Thompson* et al., 1986; *Cahill* et al., 1987; *van Bossuyt* et al., 1990). However, this hypothesis seems to be refuted by a recent report that demonstrated that both internal and external drainage reversed endotoxaemia and reduced mortality in obstructive jaundiced rats (*Diamond* et al., 1990).

### Bowel obstruction

Bowel obstruction rapidly causes systemic endotoxaemia (*Roscher* et al., 1988), as well as an increase in bacterial translocation (*Deitch* et al., 1989b; 1990d). Intestinal endotoxaemia following bowel obstruction likely results from an increase in the endotoxin concentration as a consequence of bacterial overgrowth (particularly in the small intestine) (*Roscher* et al., 1988), and a decreased barrier function of the bowel mucosa (*Roscher* et al., 1988; *Deitch* et al., 1990d). The latter is possibly related to toxic effects of extremely high endotoxin concentrations on epithelial integrity (*Roscher* et al., 1988; *Walker* and *Provaznik*, 1978).

### Inflammatory bowel disease

Severe inflammatory bowel disease may cause extensive ulceration of the small or large intestine, and may be complicated by systemic endotoxaemia (*Palmer* et al., 1980; *Fink* et al., 1988). Surprisingly, even in patients with severe damage to the bowel mucosa, septic shock is not frequently observed. It is possible that the continuous exposure to endotoxin in these patients causes tolerance to its biological effects. Alternatively, patients with inflammatory bowel disease may develop specific anti-endotoxin defence mechanisms. For example, patients with Crohn's disease frequently have high titres of anti-lipid A antibodies.

### Other conditions

Protein malnourished mice are more susceptible to endotoxin-induced bacterial translocation than controls (*Li* et al., 1989). Endotoxaemia was present in 44% of severely malnourished Thai children, and correlated with the presence of vitamin A deficiency (*Klein* et al., 1988). Systemic endotoxaemia may also be induced in monkeys by heat stress, and the occurrence of endotoxaemia predicts mortality (*Gathiram* et al., 1987).

## PHYSICAL BINDING OF ENDOTOXIN IN THE GUT

As discussed above, endotoxins are present in large quantities in the gut, derived from endogenous Gram-negative flora. In an experimental model of serotonin-induced intestinal endotoxaemia in mice, systemic endotoxaemia may be reduced by the use of kaopectate and charcoal particles that bind endotoxin in the gut. Lactulose and pectin, however, showed no effect on the endotoxaemia in the same study (*Ditter* et al., 1983). When lactulose was orally administered preoperatively to jaundiced patients, the level of postoperative portal and systemic endotoxaemia diminished (*Pain* and *Bailey*, 1986). Oral, nonresorbed antibiotics do not significantly reduce the amount of gut endotoxins, with the exception of polymyxin (*van der Waaij* et al., 1985). In fact, in mice a transient rise in the faecal endotoxin content was observed after selective bowel decontamination with streptomycin, neomycin and amphotericin B (*Rogers* et al., 1985). Prolonged administration of neomycin to humans may

however decrease the faecal endotoxin concentration. It is nevertheless difficult to explain the finding that in experimental animals kanamycin protects against endotoxaemia induced by vaso-active amines or bowel ischaemia (*Ravin* et al., 1960). Bile salts have been reported to reduce intestinal uptake of endotoxins in patients with obstructive jaundice (*Thompson* et al., 1986), and preoperative administration of sodium deoxycholate to these patients has been shown to prevent portal endotoxaemia (*Cahill*, 1983). In other conditions however, in particular septicaemia, there is yet no direct proof for the clinical usefulness of any drug that interferes with intestinal endotoxin uptake.

## TREATMENT STRATEGIES FOR SEPTICAEMIA

In the last decade, numerous studies have led to different (immuno)therapeutical approaches in septicaemia. A key finding in immunotherapy for Gram-negative septicaemia was the demonstration of therapeutic efficacy of antibodies that bind epitopes on the endotoxin core or the lipid A component in animal models of endotoxaemia and Gram-negative infection. The development of this strategy began with the production of polyclonal antisera to endotoxin core by immunising rabbits with heat inactivated mutants of *S. typhimurium* TV119, *E. coli* O111:B4 and *S. minnesota*. Because these mutants lack the immunodominant O-polysaccharides, they allow antisera to be raised against the conserved inner core determinants. Antisera obtained from immunised rabbits conferred passive protection against the dermal Shwarzman reaction caused by endotoxin derived from a wide variety of Gram-negative organisms (*Braude* and *Douglas*, 1972) and protected mice and neutropenic rabbits from lethal bacteraemia due to *E. coli*, *Klebsiella*, *Pseudomonas* and *Proteus* (*Tate* et al., 1966; *Chedid* et al., 1968; *McCabe* and *Greely*, 1972; *Ziegler* et al., 1973). Subsequently, a human polyclonal antiserum to endotoxin was developed by immunising human volunteers with the heat inactivated *E. coli* J5 mutant. In one large clinical trial, treatment resulted in a marked reduction in mortality in patients with objectively documented Gram-negative bacteraemia (*Ziegler* et al., 1982), and prophylactic use of the antiserum prevented mortality due to septic shock and reduced the morbidity in surgical patients at high risk for developing Gram-negative infections (*Ziegler* et al., 1982; *Baumgartner* et al., 1985). In patients with prolonged neutropenia however, prophylactically administered polyclonal anti-J5 serum did not reduce the number of Gram-negative bacteraemic episodes (*McCutchan* et al., 1983). For several reasons, including the variability on antibody titres and the risk of transmission of infectious agents, administration of immune serum does not seem to be practical in septic patients. Monoclonal antibodies have constant and established specificities, and can be produced in large amounts. The results from recently published large multicentre trials employing monoclonal anti-endotoxin antibodies directed against the J5 mutant of *E. coli* showed a remarkable reduction in the 28 day mortality of 37-39% in Gram-negative bacteraemic patients and 39-42% in patients with septic shock (*Gorelick* et al., 1990; 1990b; *Ziegler* et al., 1991). There were no adverse effects that could be attributed to the treatment. Thus, the efficacy and safety of monoclonal antibodies to endotoxin seems established and they

therefore have the potential of becoming the immunotherapeutic drug of choice in clinical medicine in the treatment of Gram-negative septicaemia. A major problem in treating septic patients is case selection. Of all patients that are clinically diagnosed as having Gram-negative septicaemia, only one third has Gram-negative bacteraemia, and one third is endotoxaemic. It is tempting to speculate that a rapid method of bedside endotoxin determination would be of help in selecting patients for immunotherapy .

## OUTLOOK FOR FUTURE TREATMENTS

The protective effects of TNF-antibodies were first established with a polyclonal rabbit antiserum against murine-TNF. This experiment showed a significant protection in a lethal endotoxaemia model in mice (*Beutler* et al., 1985c). Subsequent investigations in rabbits showed similar results (*Mathison* et al., 1988). Strong evidence was provided by a study which showed that monoclonal anti-TNF antibodies protected against the development of septic shock and lethal tissue injury in acutely bacteraemic baboons (*Tracey* et al., 1987). Large, multicentre trials using anti-TNF antibodies as the immunotherapeutic drug for septicaemia are currently underway. Clearly, TNF is not the only inflammatory mediator, and recently it has been shown that rabbits can be protected from the lethality of endotoxaemia by pretreatment with recombinant interleukin-1 receptor antagonist (*Wakabayashi* et al., 1991). All these treatment strategies are exciting, but their clinical efficacy has yet to be proved.

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